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VARIOUS SPOROTRICHIA DIFFERENTIATED BY THE FERMENTATION OF CARBOHYDRATES

STUDIES ON AMERICAN SPOROTRICHOSIS, 1*

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Pathogenic sporotricha have been isolated during the last fourteen years in increasing numbers, and their importance as a causative agent of various forms of diseases has been fully recognized. The exhaustive studies of De Beurmann and Gougerot¹ have added valuable information to our knowledge concerning the American sporotricha. In comparing numerous organisms which they had isolated in France with other strains from North and South America, Italy, Austria, etc., assisted by the best mycologists (Matruchot and Ramond), they attempted a classification of the genus sporotrichum. Seven different species were created, to which Langeron² recently added another. The species groups discussed by De Beurmann and Gougerot are, with the exception of the sporothrix beurmanni group, represented by one or two specimens only. This fact is of considerable importance when one remembers that this group of higher fungi is so exceedingly pleomorphic, and that neither the macroscopic nor the microscopic characteristics for all the species discussed by the two French investigators vary to such a degree that, without a detailed study, a difference could be noted. The pleomorphism of Sporothrix beurmanni on solid carbohydrate culture media, when Sabouraud's glucose agar is not used, is so frequent, and is often so inconstant and uncontrollable, that it is not surprising to note that practically all the American mycologists (Gifford, Davis, and others), who isolated and superficially studied a small number of sporotricha, refused to accept the classification of Gougerot.

In studying carefully the publications of Gougerot, of Matruchot, and others, we became convinced however that if the facts are as

* Received for publication March 15, 1915.

1. Arch. de parasitol., 1911, 15, p. 5; Kollé-Wassermann: Handbuch der pathogenen Mikroorganismen, 1913, 5, p. 2.

2. Arch. de parasitol., 1913, 16, p. 307.

stated in the publications, the writers were fully justified in creating a species *Beurmanni* in addition to the species *Sporothrix schenckii*. The differences—various types of pleomorphism and pigmentation, formation of chlamydospores, fermentation of carbohydrates—are doubtless very pronounced. De Beurmann and Gougerot compared however only one strain: the so-called *sporothrix schenckii*, which was isolated by Hektoen and Perkins in 1900 and which was considered by Hektoen to be identical with the strain isolated by Schenck in 1898.

Since 1908 Hyde and Davis,⁸ Ruediger and Hiller,⁴ Page, Frothingham and Paige,⁵ Gifford,⁶ and Trimble and Shaw,⁷ have isolated in North America numerous new strains of sporotricha from human and animal cases and have shown that the “*Beurmanni*” characteristics are not always present, as far as their, in many respects, very superficial morphologic and biologic studies permit us to conclude. Davis⁸ in particular during the last two years has pointed out that there is no reason to accept the classification of De Beurmann and Gougerot as final.

His first paper dealt with the agglutination test as a means of differentiation between the various sporotricha. The sera of immunized rabbits flocculated the spores of all the different *sporothrix* strains in the same dilutions. Inasmuch as our experience has shown that the agglutination test for *sporothrix* is only applicable for clinical purposes, and not for immunological or differential tests, and that rabbits are not very suitable for immunization with sporotricha, we feel that these methods were not practicable in deciding whether *Sporothrix beurmanni* exists in North America or not. Extensive tests which were carried out by us by means of the complement fixation and agglutination tests, using horse and dog sera, only demonstrated that the different strains which we have isolated are identical from an immunological viewpoint.

In his second paper, Davis,⁹ investigating the chlamydospore production, found that the *sporothrix schenckii* also produces on special media which are poor in nutritive substances chlamydospores just as abundantly as do the European strains. We have had ample oppor-

3. Jour. Cutan. Dis., 1910, 28, p. 321.

4. Jour. Minnesota Med. Assn., 1911, 31, p. 507.

5. Jour. Med. Research, 1910, 23, p. 137.

6. Ophth. Rec., 1910, 19, p. 573.

7. Jour. Kansas Med. Soc., 1909, 9, p. 305.

8. Jour. Infect. Dis., 1913, 12, p. 140.

9. Ibid., 1914, 15, p. 580.

tunity to study the production of chlamydo-spores and have always been able to demonstrate them on ordinary Sabouraud's medium. Even the original Hektoen strain (*Sporothrix schenckii* of Gougerot) produced in our hands, in several old cultures, the spores under discussion. The strain had been passed through young rats: Some of our equine strains frequently failed, in the first two or three generations, to produce chlamydo-spores, and some of the strains still produce them in very small numbers only. On the medium of Davis, however, twelve strains selected at random produce the spores in abundance, so that we are quite prepared to confirm the statements of Davis and also to corroborate his conclusion that the presence of chlamydo-spores does not justify the identification of the strain as *Sporothrix beurmanni*.

In addition to these two points of differentiation, the pleomorphism, the pigmentation, and the fermentation properties of the sporothrix are considered in the classification of De Beurmann and Gougerot.

Pleomorphism.—The first feature has been entirely ignored in all American publications on pathogenic sporotricha. Concerning the sporothrix schenckii De Beurmann and Gougerot write as follows:

Unité et Pléomorphismes.—Cette étude n'est pas signalée par les auteurs américains. Les pléomorphismes des cultures étudiées (échantillon Hektoen-Gougerot) sont presque nuls. A peine note-t-on sur quelques tubes de cultures en milieux pauvres une tendance du voile à devenir lisse et sur certains tubes de pomme de terre glycosée-peptones un développement exubérant des piquants, un poudrage blanc-mat; les piquants très touffus, souvent réticulés à leur base, atteignent jusqu'à 10 et 12 millimètres de longueur et 1 à 2 millimètres de largeur; ils peuvent envahir tout le tube et s'accoller au verre en face de la culture.

En réalité, nos cultures de *Sporotrichum Schencki* (échantillon Hektoen-Gougerot) se sont montrées d'une fixité remarquable à l'inverse de celles de *Sporotrichum Beurmanni*.

The publications of Hyde and Davis,³ Hamburger,¹⁰ Trimble and Shaw,⁷ and Albert and Grover,¹¹ do not record observations of pleomorphism. Some of their strains, according to the descriptions given and according also to the pictures accompanying the articles, show clearly that it occurs also in American strains. Our observations on thirty-five strains have demonstrated that pleomorphism is a characteristic feature of most of the American strains and that it is just as common as has been described in *Sporothrix beurmanni*. On maltose agar particularly, one notices pleomorphism very regularly, just as De

10. Jour. Am. Med. Assn., 1912, 59, p. 1590.

11. Iowa Med. Jour., 1913, 19, p. 428.

Beurmann and Gougerot pointed out as early as 1906. The original strain Hektoen has, in our experience, a rather fixed growth, which can be stimulated to a pleomorphic surface growth (smooth, not folded and shiny, having powdery, dull growth) only by repeated cultivation on maltose agar.

In another publication we will discuss and illustrate this condition in detail. We will emphasize however that our strains, isolated and kept on Sabouraud's media, behave just as the Beurmanni strains with regard to the pleomorphic growth. The pleomorphic growth, if absolutely typical and constant, would indicate that the recently isolated American (human and animal) strains are of the "Beurmanni" type.

Pigmentation.—The second feature of distinction, according to De Beurmann and Gougerot, "pigmentation," is claimed to be constantly absent, particularly on potato media. The colonies remain snow-white or grayish, even when old and dry, in cultures of *Sporothrix schenckii*-Hektoen-initial strain. On the other hand, the Beurmanni strains become dark-brownish; some strains change very rapidly to a deep black. These statements of De Beurmann and Gougerot are surprising, inasmuch as Schenck and Hektoen and Perkins show clearly that a brownish, yellowish pigmentation of the old cultures also occurred; that no deeper black was observed is perhaps due to the fact that no glucose or glycerin had been added to the potatoes, as was the case in the tests of De Beurmann and Gougerot. This is not the occasion to discuss the possibility that the original Hektoen strain, by growth on improper media during the six years since its isolation, had been entirely modified. In our hands the Hektoen strain formed pigment on glycerin-peptonized potatoes, so that it could be classified with the B-Beurmanni strains.

The recently isolated strains of Davis, Hamburger, Trimble and Shaw, Gifford, and Page, Frothingham and Paige, all show distinct pigmentation. Trimble and Shaw⁷ report, for example, that their cultures showed a "turning brown, deepening to black with age." The plate accompanying the publication of Page, Frothingham and Paige⁵ shows a most decided blackening of the potato cultures, so that one would not hesitate to diagnose the strain, from these drawings, as a type of *Sporothrix beurmanni*. All these facts make it apparent that the pigmentation is not always absent in the "Schenckii strains," and in the light of the work of De Beurmann and Gougerot, the bac-

teriolgists who have isolated sporotricha in this country since 1910 could not safely diagnose their strains as *Sporothrix schenckii*. The statement of Gifford (which, according to our experience, is correct) that an American pigmented sporothrix strain could be turned to an unpigmented Schenckii-like strain when grown on lactose agar, or influenced by other means, does not affect the question of pigmentation on the differential media like glucose agar and potato, and it would be better if such attempts of mutation were not used to complicate the already existing difficulties of classification.

Strains of the α , β , and γ type, as recognized and clearly defined by De Beurmann and Gougerot, have been isolated by us, as will be shown later. However, the pleomorphism of pigmentation is not absolutely constant, and as soon as one studies a large number of strains, one will agree with De Beurmann and Gougerot, who state that an α strain can be easily changed into a β or γ strain, and vice versa. This characteristic cannot be used therefore for differentiation.

Fermentation of Carbohydrates.—The third feature, the fermentation of carbohydrates, is constantly reported by De Beurmann and Gougerot as being a distinctive phenomenon between *Sporothrix schenckii* and *Sporothrix beurmanni*. Even when one is confronted with the fact that the method of biochemical tests in these groups of organisms is not as easy and quick a procedure as it is in the colon-paratyphoid group, a comparative study to verify the statements of De Beurmann and Gougerot suggests itself.

Gougerot and Blanchetière¹² reported that all the Beurmanni strains studied by them fermented saccharose but not lactose; the original Hektoen strain of *Sporothrix schenckii* hydrolyses and ferments lactose, but not saccharose. Schenck¹³ and Hektoen and Perkins¹⁴ report only that in the fermentation-tubes no gas is liberated by the growth of the sporotricha in lactose, glucose, and saccharose broth; there are no statements as to the acid production. Page, Frothingham and Paige⁵ report that their two equine strains produced only acid in glucose, galactose, and dextrin serum-water-litmus media; but as to the last two carbohydrates, they desire further confirmation.

In 1913 a preliminary test was carried out by one of us (K. F. M.), using a somewhat modified medium from the one recommended by Blanchetière and Gougerot, and selecting from our collection of 35

12. Compt. rend. Soc. de biol., 1909, 16, p. 202.

13. Bull. Johns Hopkins Hosp., 1893, 9, p. 286.

14. Jour. Exper. Med., 1909, 5, p. 77.

strains, 15 strains which showed, on Sabouraud's media, more or less striking differences.

The strains were all grown in small Erlenmeyer flasks (10 c.c.) for sixty to ninety days, and by means of cork floaters a surface growth was obtained. Only those flasks on which a good, thick, felt-like surface growth was present, were considered suitable for a final reading. As an indicator, only litmus was used. The cultures were kept in a cupboard, in the space situated between the double doors of an incubator-room; the temperature varied from 26-32 C. On account of the number of flasks and the lack of available space, only seven carbohydrates—glucose, lactose, maltose, saccharose, mannite, galactose, levulose, and glycerin—were tested. Only Merck purest chemicals were used. The results are compiled in Table 1.

All strains fermented decidedly glucose with acid production but no gas. No strain fermented lactose or mannite. This result is surprising, inasmuch as the *sporothrix schenckii* should ferment the lactose. With regard to the other carbohydrates, no uniform results were obtained. Most of the strains, with the exception of an atypical one, fermented levulose; only seven fermented saccharose; maltose was inverted by only five strains; galactose was changed by practically the same strains. As far as tested, glycerin was fermented in the same irregular manner as noted for the other strains. These results confirm the statements of Blanchetière and Gougerot, that pathogenic sporotricha—particularly *Sporothrix beurmanni*—ferment glucose, maltose, saccharose, galactose, levulose and glycerin, but not lactose and mannite. However, in our series less than 40 percent of the strains fermented the carbohydrates mentioned in a regular manner; most of the strains attacked only glucose and levulose. The strains fermenting saccharose, therefore, would be classified according to Blanchetière and Gougerot with the *sporothrix beurmanni*. These developed marked fermentative activities also in the other carbohydrates. Unfortunately, the test strain *Sporothrix beurmanni* obtained from D. J. Davis failed to give the reactions claimed by Blanchetière and Gougerot.

The amount of acid produced was not tested in detail, but it varied considerably. At the first glance it is apparent that those strains which were perpetuated for several generations on Sabouraud's medium fermented more freely the different carbohydrates than the more recently isolated ones. This point, however, is not constant. Some strains like B and U are decided *Sporothrix beurmanni* strains in their morphological appearance and growth on the various test media. Strain B was identified by Dr. de Beurmann, in a personal communication to one of us, as a *sporotrichum beurmanni*. This superficial test shows only that some *sporothrix* strains will ferment various carbohydrates; the results, however, are irregular, inconstant, and of no diagnostic value. The strain T proved, on further examination, to be a blastomycotic sporotrichum, as first suspected.

In 1914, after most of the strains had been kept on Sabouraud's medium for many months, it was thought best to test again the fermentative action of a series of sporotricha strains, selected at random, growing the same for a longer period and in large (1 L. and 500 c.c.) flasks. The cultures were kept at room temperature, without tin-foil or rubber-cap covering of the cotton plugs. Furthermore, it was considered advisable to use the medium recommended for such tests by Blanchetière. Also, a strain identified by the Institut Pasteur as *Sporothrix beurmanni* was used as a control. The acid production was determined by titration with N/20 NAOH after 68, 202, and 270 days, respec-

TABLE 1
FERMENTATION BY SPOROTRICHA

Carbohydrate, 4 percent; litmus, 5 percent; peptone, 1 percent solution.

Strains *	Glucose	Lactose	Saccharose	Maltose	Mannite	Galactose	Levulose	Glycerin
Sp. schenckii (D. J. Davis) (Hektoen- initial) Hektoen-Gougerot.....	+	0	0	0	0	0	+	+
Sp. Beurmanni (D. J. Davis) (French strain).....	+	0	0	0	0	0	+	+
Strain A/7.....	+	0	+	+	0	+	+	+
Strain AB/4.....	+	0	+	+	0	+	+	+
Strain B/8.....	+	0	+	+	0	+	+	+
Strain C/7.....	+	0	+	+	0	+	+	+
Strain D/5.....	+	0	0	+	0	0	+	—
Strain E/5.....	+	0	+	+	0	+	+	—
Strain F/4.....	+	0	+	+	0	0	+	—
Strain G/4.....	+	0	+	0	0	0	+	—
Strain K.....	+	0	+	0	0	0	+	—
Strain L.....	+	0	0	0	0	0	+	—
Strain M.....	+	0	0	0	0	0	+	—
Strain T.....	+	0	0	0	0	0	+	—
Strain U/4.....	+	0	+	0	0	0	+	0

* The history of these strains is given in the appendix.

The sign + + + means decidedly acid, bright red; + + acid, purplish; 0 unchanged; — not tested. The figures indicate the acidity determined by titration.

tively, of cultivation. Only four carbohydrates (Merck's chemicals), glucose, lactose, saccharose, and starch, were used.

The results are tabulated in Table 2. The figures indicate the actual amount of acid produced after having deducted the figure indicating the acidity in the control flasks.

TABLE 2
FERMENTATION BY SPOROTRICHIA

Strains	Lactose		Saccharose		Glucose	Starch
	68 Days	270 Days	68 Days	270 Days	202 Days	202 Days
Sp. beurmanni	0.11	.6	0.15	0.8	4.35	4.35
Sp. hamburger00	0.5	0.14	0.4	4.25	1.05
Sp. schenckii (Hektoen- Gougerot) (Hektoen- initial)00	0.25	0.20	0.45	4.55	1.05
B 1200	0.35	0.84	3.0	2.65	2.95
C 1000	0.1	0	0.15	2.50	3.95
F 6	0.13	0.4	0.28	4.05	3.20	0.65
K 7	0.35	0.6	0.58	2.6	2.20	0.8
L 7	0.13	0.45	0.08	0.35	3.15	2.05
Z 6	0.10	0.3	0.13	3.4	4.60	1.95
DD 5	0.12	0.55	0.27	2.4	3.1	2.7
AB 1000	0.55	0.20	2.9	3.2	0.55
CC 4	0.03	..	0.08	3.45	3.8	1.4

The results show that all strains (12) tested, decidedly ferment glucose. The acid produced was, as previous tests had shown, in the main lactic acid. Lactose was not attacked by the strains after sixty-eight days' cultivation; after 270 days, however, every strain produced traces of acid. The nature of it has not as yet been determined, but it appears from other tests that similar small amounts of acid are produced by sporotricha in media not containing carbohydrates; this fact is observed in similar tests with other organisms (typhoid, anaerobes, etc.). It is therefore apparent that the sporothrix schenckii strains (Sporothrix Hamburger and Schenckii—original—Hektoen) did not ferment lactose. These findings are in contradiction to those of Blanchetière and Gougerot. The fermentation of saccharose and starch was irregular. Only seven strains fermented saccharose, the control strain of Sporothrix beurmanni failing to produce a marked acidity which could be considered as a fermentation of the carbohydrates. Following the classification of Blanchetière and Gougerot, the strains B₁₂, F₆, K₇, Z₆, DD₅, AB₁₀, and CC₄, would be true Sporothrix beurmanni strains. The acid production in starch is not marked for two strains only; the growth in these flasks was poor compared with the other cultures. The absence of hydrolysis in these flasks and the subsequent absence of glucose explains, in our opinion, the dis-

crepancies. Blanchetière and Gougerot found fermentation of starch to occur for both *Sporothrix beurmanni* and *schenckii*.

In considering these results purely from a differential diagnostic viewpoint, it is quite evident that it cannot be used for this purpose, and the fermentation of carbohydrates is just as little a criterion of the type of sporotrichum as is the absence of pleomorphism and the chlamydospore formation.

Blanchetière and Gougerot tested only six varieties of sporotricha in saccharose, of which only four fermented this carbohydrate. The report gives no information as to the classification of the varieties. It is mentioned however that *Sporothrix schenckii* was one of the two varieties which did not ferment saccharose.

These two investigators expressed the opinion that they were unable to state whether the fermentative reactions were entirely fixed for all the *Beurmanni* strains, or whether, on account of the tendency to pleomorphism, certain variations were possible. The findings of Greco,¹⁵ with a strain which he named "*Sporothrix schenckii-beurmanni*," isolated in Uruguay, are similar to ours and therefore prove conclusively that the fermentative activities of the sporotricha are not fixed. The strain of Greco did not ferment mannite, lactose, and saccharose.

During the course of these observations it was noted that the fermentative activities showed some relation to the growth of the strains on Sabouraud's medium. The amount of pigment, and the time of its appearance, correspond in some respects to the ability to ferment saccharose.

De Beurmann and Gougerot distinguished, according to the pigmentation on Sabouraud's medium, a sporotrichum α , β , and γ . The type " α " is, ordinarily, very poorly pigmented; it remains white or light-brownish; the pigment appears slowly, and only portions or segments of the colonies are tinged. The " β " type reaches a chocolate-brownish color and pigments slowly also. The " γ " type changes from a dirty gray color to a deep ebony black in four to five days. The tinge is always deep black, or brownish-black, with brownish or steel-bluish reflections. The distinction of these three types is not absolute and cannot be used for differentiation. In our experience, however, the ability to form pigment remained fairly constant for the strains studied by us (with one exception) for the period of four years during which we made observations. The classification of the various sporo-

15. Argentina Med., 1907, p. 699.

tricha together with their acid production in saccharose, is shown in Table 3.

TABLE 3
CLASSIFICATION AND ACID PRODUCTION OF SPOROTRICHA

		68 Days	270 Days
"α" type..	Strain B	0.84	3.0
	Strain F	0.28	4.05
	Strain Z	0.13	3.4
	Strain AB	0.20	2.9
"β" type..	Strain K	0.58	2.6
	Strain CC	0.08	3.45
	Strain DD	0.27	2.4
	Strain C	0.0	0.15
"γ" type..	Strain L	0.8	0.35

The strains producing only small amounts of pigment proved to be the most vigorous saccharose fermenters. This fact is proven in a very interesting observation. Strain C fermented, according to Table 1, saccharose. In the last test, however, it failed to produce a marked amount of lactic acid. According to our records the strain was a β type when isolated; passed, later, through several animals, it changed entirely to a γ type. The original culture having been lost, one of the subcultures of the animal-passage was used without further consideration. The result is clearly shown; the strain does not ferment saccharose in the second test. In our opinion this observation is further proof that the carbohydrate fermentation is inconstant and dependent upon numerous factors, of which animal passage seems to be one. The strains of Page, Frothingham and Paige being of the γ type behaved therefore correctly in not fermenting the saccharose.

For the existing relation of pigmentation to fermentation, we are at present unable to offer any definite explanation. Some additional experiments are in progress which we hope will throw some light on this phase of the chemical activity of the sporotricha.

CONCLUSIONS

The differentiation of pathogenic sporotricha into two distinct species, by means of the fermentation of carbohydrates, is impossible. The reactions are not fixed and are as inconstant as the many variations noted in the formation of chlamydospores and, frequently, in pleomorphism. There does exist however an apparent relation between the pigmentation of the sporotrichum strains and the ability of these strains to ferment saccharose. The α and β types are the most active fermenters.

This and other evidence, which will be presented elsewhere, make it apparent that the American sporotricha—of which we studied thirty-five strains—have, in many respects, type characters in common with *Sporothrix beurmanni*. In the light of De Beurmann's and Gougerot's

work, some of the American strains are doubtless *Sporothrix beurmanni*, and it is not permissible to call such strains "*Sporothrix schenckii*" merely for the sake of simplicity. The discussion of De Beurmann and Gougerot¹⁶ on this subject can now also, in our opinion, be satisfactorily closed, namely: that *Sporothrix schenckii*, Hektoen-Gougerot strain, is an absolutely fixed type. The true *sporothrix schenckii* is represented however by all of the recently isolated strains. Inasmuch as most of these strains are undoubtedly identical with *Sporothrix beurmanni*, the *sporothrix schenckii* is identical with the *sporothrix beurmanni*.

The American strains of pathogenic sporotricha are therefore best classified as one species: *Sporothrix schenckii-beurmanni* (as suggested by Greco).

APPENDIX

HISTORY OF THE STRAINS

Sp. *beurmanni*: Original tube was received from Institut Pasteur, Paris, March, 1914.

Sp. *schenckii*: Original tube was received from Dr. D. J. Davis, Chicago, 1913 and 1914. This is the so-called "Hektoen-Gougerot-initial" strain.

Sp. *schenckii* (Hamburger): Original tube was received from Dr. D. J. Davis, Chicago.

Strain "A": Original was isolated by K. F. Meyer from pus collected from a horse with so-called epizootic lymphangitis, October, 1911.

Strain "AB": Original was isolated by K. F. Meyer, October, 1911, from experimental horse, "Nancy," January, 1912.

Strain "B-12": Original was isolated by K. F. Meyer from pus collected from a horse with so-called epizootic lymphangitis, April 2, 1912.

Strain "C-10": Ditto; by K. F. Meyer; ditto, May 23, 1912.

Strain "CC": Original was isolated from a human case of sporotrichosis, April 30, 1913, by K. F. Meyer.

Strain "D-5": Original tube was received from Dr. D. J. Davis, Chicago, December, 1911.

Strain "DD-5": Original was isolated by K. F. Meyer from equine case of sporotrichosis, June 4, 1913.

Strain "E-5": Original was isolated by K. F. Meyer, collected from a horse with so-called epizootic lymphangitis, July, 1912.

Strain "F-6": Ditto; July 1, 1912.

Strain "G-4": Original tube was received from Dr. G. F. Ruediger, North Dakota, July, 1912.

Strain "K-7": Original was isolated by K. F. Meyer from a human case of sporotrichosis in Strattonville, Pa., July 1, 1912.

Strain "L-7": Original was isolated by K. F. Meyer from an equine case of sporotrichosis July 19, 1912.

Strain "M": Original was isolated by K. F. Meyer from pus collected from a mule with so-called epizootic lymphangitis, January 3, 1913.

Strain "T": Original, a blastomycotic strain of sporothrix, was isolated by K. F. Meyer from an experimental case of sporotrichosis, August, 1912.

Strain "Z-6": Original was isolated by K. F. Meyer from an equine case of sporotrichosis, April 14, 1913.

16. Bull. et mém. Soc. Med. d. hôp. de Paris, 1908, 26, p. 9.